INVESTIGATION OF THE PHYSICAL PROPERTIES OF CHOLESTERYL ESTER LIQUID CRYSTAL AND THE INTERACTION WITH CELLS

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A thesis submitted in
fulfilment of the requirement for the award of the
Degree of Master of Electrical Engineering

Faculty of Electrical and Electronic Engineering
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JANUARY 2016

For my dearest mother, father, husband and family for their encouragement and blessing...

To my beloved friend and their support and caring......

-Never tired and give up to gain knowledge and life is a journey of learning-

ACKNOWLEDGEMENT

Alhamdulillah, I am grateful to ALLAH S.W.T the most merciful and the most compassionate for the guidance and knowledge bestowed upon me, for without it I would not been able to come this far. Peace be upon him, Muhammad the messenger of god.

I would like to express my gratitude to honourable Assoc. Prof. Dr Soon Chin Fhong, my supervisor of Master's project. Under her supervision, many aspects regarding this project has been explored and with the knowledge, idea and support received from her. I also would like to thank my co-supervisor Dr Hatijah Basri for sharing her knowledge to me.

Many thanks and grateful to the Malaysia Ministry of Higher Education for research funding support (FRGS Phase 1 Vot No. 1050) and also Universiti Tun Hussein Onn Malaysia for providing post graduate incentive research grant (GIPS Vot 1111).

Finally, I would like to dedicate my gratitude to my parents, my family, my husband and friends who helped me directly and indirectly in the completion of this project. This encouragement and guidance mean a lot to me. Their sharing and experience foster my belief in overcoming every obstacle encountered in this project.

Guidance, co-operation and encouragement from all people above are appreciated by me in sincerely. Although I cannot repay the kindness from them, I would like to wish them to be well and happy always.

LIST OF ASSOCIATED PUBLICATIONS

Journals

1. Chin Fhong Soon, Wan Ibtisam Wan Omar, Rebecca F. Berends, Nafarizal Nayan, Hatijah Basri, Kian Sek Tee, Mansour Youseffi, Nick Blagden, Morgan Clive Thomas Denyer (2014) "Biophysical characteristics of cells cultured on cholesteryl ester liquid crystal," Micron, Vol. 56, pp. 73-79. Published by Elsevier B.V.

Conference Proceeding:

1. Chin Fhong Soon, Wan Ibtisam Wan Omar, Nafarizal Nayan, Hatijah Basri, Martha Bt. Narawi, Kian Sek Tee (2013) "A Bespoke Contact Angle Measurement Software and Experimental Setup for Determination of Surface Tension." Proceeding of 4th International Conference on Electrical Engineering and Informatics (ICEEI), Procedia Technology. Vol. 11, pp. 487-494. Published by Elsevier B.V.

2. Wan Ibtisam Wan Omar, Chin Fhong Soon (2014) "Critical Surface Tension of Cholesteryl Ester Liqiud Crystal." Proceeding of Joint International Conference on Nanoscience, Engineering and Management (BOND 21), Bayview Beach Resort, Batu Ferringhi, Penang, Malaysia, 19 – 21 August 2013, Advance Materials Research. Vol. 925, No. 4, pp. 43-47. Trans Tech Publications, Switzerland.

3. Wan Ibtisam Wan Omar, Chin Fhong Soon (2015) "The Time Based Study of Cell Morphology Using Atomic Force Microscopy." Proceeding of 5th International Conference on Biomedical Engineering (IFMBE), Vietnam, 16-18 June 2014, Vol. 46, pp. 227-230. © Springer International Publishing Switzerland.

LIST OF AWARDS

1. **Silver Medal in Research & Innovation Festival 2012 (R & I Fest UTHM)**

   Chin Fhong Soon, Hatijah Basri, Kian Sek Tee, Wan Ibtisam Wan Omar, Morgan Denyer. "Simple Apparatus and a Contact Angle Measurement Software for Automatic Computation of Surface Tension."

2. **Gold Medal in Persidangan dan EXPO Ciptaan Institusi Pengajian Tinggi Antarabangsa 2013 (PECIPTA)**

   Chin Fhong Soon, Wan Ibtisam Wan Omar, Hatijah Basri, Morgen Denyer. "An Integrated System For Measurement and Mapping of Single Cell Traction Force."

3. **Silver Medal in Research & Innovation Festival 2013 (R & I Fest UTHM)**

   Thong Kok Tung, Wan Ibtisam Wan Omar, Rosliza Mohamad Zin, Yap Huing Yin. "Scaffoldless Technique to Culture 3D Keratinospheroids."

ABSTRACT

Cholesteryl ester liquid crystals (CELC) were demonstrated with application in biosensing and microtissue regeneration. The affinity of the cells to this liquid crystal is unclear and required further investigation. This study focused on characterising the physical properties of CELC and interaction of human keratinocytes with CELC. The physical properties of CELC were characterised by a custom built contact angle measurement system and bubble pressure measurement apparatus. Other methods such as pendant drop were applied to determine the critical surface tension of the CELC. Then, the characterization of the CELC was continued by using Differential Scanning Calorimeter (DSC), X-ray Diffraction (XRD), Polarising Microscopy (POM) and Fourier Transform Infrared Spectroscopy (FTIR). Nonetheless, the morphology of cells interaction with CELC after it reached confluency was studied using Field Emission Scanning Electron Microscopy (FESEM) and non-contact mode of Atomic Force Microscopy (AFM). The results showed that the critical surface tension of the liquid crystal using contact angle was 37.5 mN/m and the surface tension measured using pendant drop method was found to be 23.6 mN/m. Both results indicate that the surface of the liquid crystal was moderately hydrophobic. From the DSC, CELC was found stable at room and incubator temperature. From XRD results, the compound of CELC interacts in cell culture media self-assembles into lyotropic layer. POM and FTIR analysis showed CELC after immersion in media displayed lyotropic smectic phases. The AFM and FESEM images indicated good adhesion of cells on the CELC. This research thus showed that the hydrophilic layers of lyotropic phase of cholesteryl ester liquid crystal were demonstrated with biophysical properties that support the adhesion of cells.

ABSTRAK

Kristal cecair kolesterlat ester (CELC) telah menunjukkan aplikasi dalam biosensor dan pertumbuhan semula tisu mikro. Pertalian sel-sel terhadap kristal cecair ini adalah tidak jelas dan memerlukan kajian lanjut. Kajian ini memberi tumpuan kepada pencirian sifat-sifat fizikal CELC dan interaksi CELC dengan keratinosit manusia. Sifat fizikal CELC dicirikan dengan menggunakan sistem pengukuran dan kaedah pengukuran tekanan gelembung sudut yang telah direka . Kaedah-kaedah lain seperti '*pendant drop*' telah digunakan untuk menentukan ketegangan permukaan kritikal CELC itu. Pencirian CELC diteruskan dengan menggunakan mesin Kalorimeter imbasan perbezaan (DSC), difraksi sinar-X (XRD), mikroskop optis berkutub (POM) dan perubahan fourier infra-merah (FTIR). Morfologi sel selepas interaksi dengan CELC dikaji menggunakan mikroskop elektron pengimbas pancaran medan (FESEM) dan mod tanpa sentuh mikroskop daya atomik (AFM). Keputusan menunjukkan ketegangan permukaan kritikal kristal cecair menggunakan sudut sentuh adalah 37.5 mN/m dan ketegangan permukaan dengan menggunakan kaedah '*pendant drop*' ialah 23.6 mN/m. Kedua-dua keputusan menunjukkan bahawa permukaan kristal cecair adalah sederhana hidrofobik. Daripada keputusan DSC, CELC didapati stabil pada suhu bilik dan inkubator. Keputusan XRD menunjukkan, apabila CELC berinteraksi dalam sel media, ia bertukar sendiri ke lapisan lyotropic. Analisis POM dan FTIR menunjukkan CELC selepas rendaman dalam media, memaparkan fasa smectic '*lyotropic*'. Gambar-gambar dari AFM dan FESEM menunjukkan sel melekat dengan baik pada CELC tersebut. Kajian ini sekali gus telah membuktikan bahawa lapisan hidrofilik fasa *lyotropic* cecair kolesterlat ester kristal mempunyai ciri-ciri biofizikal yang menyokong lekatan sel.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

SYMBOL		DESCRIPTION
α	-	Radius of curvature at apex drop
Å	-	Angstrom
B	-	Drop shape factor
C	-	Carbon
cm	-	Centimetre
cm-1	-	Wavenumber
°C	-	Degree celcius
d	-	Interplanar spacing
γ or σ	-	Surface tension
$\Delta\rho$	-	Difference between densities
Φ	-	Coordinate
θ	-	angle
F	-	Free energy
H	-	Hidrogen
I	-	Intensity
l	-	litre
g	-	Gravity
N	-	Nitrogen
%	-	Percentage
O	-	Oxygen
P or p	-	Pressure
R	-	Radius
T	-	Transmission
Tg	-	Glass Transition
Tm	-	Melting points
λ	-	Wavelength
m	-	metre

mA	-	Mili Amp
ml	-	Mili litre
mg	-	Mili gram
mN/m	-	Mili Newton per metre
mM	-	Micro molar
kW	-	Kilo Watt
kV	-	Kilo Volt
µ	-	micro

LIST OF ABBREVIATIONS

SYMBOL		DESCRIPTION
5CB	-	Pentylcyanobiphenyl
8CB	-	4′-n-octyl-4-cyano-biphenyl
ATR	-	Attenuated Total Reflectance
AFM	-	Atomic Force Microscopy
CELC	-	Cholesteryl Ester Liquid Crystal
CST	-	Critical Surface Tension
DMEM	-	Dulbecco’s Modified Eagles’s Medium
DMSO	-	Dimethyl Sulfoxide
DNA	-	Deoxyribonucleic Acid
DSC	-	Differential Scanning Calorimeter
DSCG	-	Disudium Cromoglycate
ECM	-	Extracellular Matrix
EG	-	Ethylene Glycol
FESEM	-	Field Emission Scanning Electron Microscope
Fmoc	-	Fluorenylmethyloxycarbonyl
FTIR	-	Fourier Transform Infrared Spectroscopy
GND	-	Ground
GUI	-	Graphic User Interface
HaCaTs	-	Human Keratinocytes
HBSS	-	Hanks Balance Salt Solution
IPA	-	Isopropyl alcohol
IR	-	Infrared
LC	-	Liquid Crystal
LCD	-	Liquid Crystal Display
LCPA	-	Liquid Crystal Pixel Array
LED	-	Light Emitting Diode
LLC	-	Lyotropic Liquid Crystal
MBBA	-	4-methoxybenzylidene-4’-n-butylaniline

MMA	-	Methylmethacrylate
MTT	-	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
OES	-	Optical Emission Spectroscopy
PEGA	-	Thermolysinpolyethylene Glycol Acrylamide
POM	-	Polarizing Optical Microscopy
PSPD	-	Position Sensitive Photo Diode
PWM	-	Pulse Width Modulation
SD	-	Standard deviation
TV	-	Television
UV	-	Ultraviolet
UTHM	-	Universiti Tun Hussein Onn Malaysia
V	-	Volt
Vis	-	Visible
XRD	-	X-ray Diffraction

CHAPTER 1

INTRODUCTION

1.1 Project background

Liquid crystal (LC) is a new class of biomaterial that gained attention for usage in biomedical engineering. They provide means for label free observation of biological phenomenon such as non-toxic thermotropic liquid crystal overlaid with matrigel that had been used in recognizing restructuring of mammalian cells [1, 2]. Recently, a research found that [3], cholesteryl ester liquid crystals (CELC) containing cholesteryl moieties may have suitable biological affinity and also enable cell adhesion and proliferation without the need for pre-treatment with extracellular matrix molecules.

Due to the compact molecular arrangement, liquid crystal was shown to have high resolution on sensing ability in detecting localized cell exerted force and could be used for cell traction force sensor [4]. This is an important property that may reveal the potential of liquid crystal in a biomaterial to function on inert surface. Cell-compatible materials are very important in many biomedical applications [5-7]. Moreover, the cholesterol derivative in the liquid crystal was a well known mesogenic nature with their potential to self order into liquid crystalline substances.

However, the use of liquid crystal in biological study has not been fully explained. In 1857, Mettenheimer used a polarising light microscope and observed double refractility and black formed cross image composed of cholesterol from blood. Researchers proved that, occurrence of anisotropic myelin-like bodies in fatty streaks of the atheromatous lesions of the aorta of man [8]. The anisotropic basic structure has been interpreted as that of liquid crystal. In our living system, liquid crystalline order of molecule occurs in degenerating tissues of liver, spleen, lung and thyroid in the form of small spheres which appear under a conventional light microscope as highly retractile and with double contours at their edges having the inner ring brighter than the outer ring [8].

This type of liquid crystal contains cholesteryl moieties which have affinity for cell membranes and have the abilities to change their properties. Cholesteryl moieties homeostasis is an important element in cellular membrane traffic and cell survival [9]. Therefore, this type of biomaterial is attractive as substrates because it could provide universally fundamental molecule to mammalian cells. Cholesteryl ester are considered to be easily biodegradable thus making the system suitable for the preparation of tissue engineering that sustain cell proliferation and migration [10]. The advantage of using liquid crystals as the biomaterial is it being non toxic and sensitive to biological interactions [11].

In addition, in order to study the biocompatibility of a biomaterial, surface energy property and thermal stability of a biomaterial are important factors in attracting the adhesion of cells and may affects cell surface interface [12]. Hence, finding surface tension of CELC was an important factor in this study. Moreover, biomaterials are majorly reliant on the surface energy [13] and stable over certain temperature. However, from the perspective of biophysical compability, little publication has been studied on the physical properties of liquid crystal. Through this study, synthesis and characterisation of surface properties CELC to interact with human keratinocytes (HaCaT) cells were reported. Furthermore, surface properties of CELC materials that promoted cell affinity were presented in this thesis.

1.2 Problem statement

In liquid crystal based biosensor development, an exposure to cell culture media is unavoidable and this may alter the surface properties of the liquid crystals over time. After immersion in cell culture media, the hydrophilic head and hydrophilic tails of the liquid crystal molecules would reoriented to interface with the water at the surface of the bulk liquid crystal as reported in [4]. However, the wettability of the surface or the surface tension of these liquid crystals in attracting the adhesion of cells have not been clearly identified and understood. Furthermore, the chemical properties of the CELC used in cell culture remain unknown. Although new applications of liquid crystals have been revealed [4], little is known about the

REFERENCES

[1] Y.-Y. Luk, S. F. Campbell, N. L. Abbott, and C. J. Murphy, "Non-toxic thermotropic liquid crystals for use with mammalian cells," *Liquid crystals,* vol. 5, pp. 611-621, 2004.

[2] S. J. Woltman, G. D. Jay, and G. P. Crawford, "Liquid-crystal materials find a new order in biomedical applications," *Nature materials,* vol. 12, pp. 929-938, 2007.

[3] C. F. Soon, M. Youseffi, N. Blagden, and M. Denyera, "Influence of Time Dependent Factors To The Phases and Poisson's Ratio of Cholesteryl Ester liquid crystals," *Journal of Science and Technology,* vol. 1, 2011.

[4] C. F. Soon, M. Youseffi, R. F. Berends, N. Blagden, and M. C. T. Denyer, "Development of a novel liquid crystal based cell traction force transducer system," *Biosensors and Bioelectronics,* vol. 1, pp. 14-20, 2013.

[5] J. H. Lee, H. W. Jung, I.-K. Kang, and H. B. Lee, "Cell behaviour on polymer surfaces with different functional groups," *Biomaterials,* vol. 9, pp. 705-711, 1994.

[6] K. Y. Lee and D. J. Mooney, "Alginate: properties and biomedical applications," *Progress in polymer science,* vol. 1, pp. 106-126, 2012.

[7] R. Landers, A. Pfister, U. Hübner, H. John, R. Schmelzeisen, and R. Mülhaupt, "Fabrication of soft tissue engineering scaffolds by means of rapid prototyping techniques," *Journal of materials science,* vol. 15, pp. 3107-3116, 2002.

[8] Y. Hata, J. Hower, and W. Insull Jr, "Cholesteryl Ester-Rich Inclusions from Human Aortic Fatty Streak and Fibrous Plaque Lesions of Atherosclerosis: I. Crystalline Properties, Size and Internal Structure," *The American journal of pathology,* vol. 3, pp. 423, 1974.

[9] R. Y. Hampton, "Cholesterol homeostasis: ESCAPe from the ER," *Current Biology,* vol. 8, pp. R298-R301, 2000.

[10] J. J. Hwang, S. N. Iyer, L.-S. Li, R. Claussen, D. A. Harrington, and S. I. Stupp, "Self-assembling biomaterials: Liquid crystal phases of cholesteryl oligo(l-lactic acid) and their interactions with cells," *Proceedings of the National Academy of Sciences,* vol. 15, pp. 9662-9667, 2002.

[11] C. F. Soon, M. Youseffi, R. F. Berends, N. Blagden, M. C. T. Denyer, B. L. Samira, and A. J. Farideh, "Characterization and Biocompatibility Study of Nematic and Cholesteryl Liquid Crystals," *Proceedings of the World Congress on Engineering,* vol. 2, pp. 1-4, 2009.

[12] B. O. Palsson and S. N. Bhatia, *Tissue Engineering* Upper Saddle River, NJ, US: Pearson Prentice Hall, 2004.

[13] B. D. Ratner, A. S. Hoffman, F. J. Schoen, and J. E. Lemons, *Biomaterials science: an introduction to materials in medicine,* New York: Academic press, 2004.

[14] I.-C. Khoo, *Liquid crystals*: Wiley-Interscience, 2007.

[15] S. N. I. Julia J. Hwang, Li-Sheng Li, Randal Claussen, Daniel A. Harrington, Samuel I. Stupp, "Self-assembling biomaterials: Liquid crystal phases of cholesteryl oligo(L-lactic acid) and their interactions with cells," 2002.

[16] H. Stegemeyer, *Liquid Crystal*, New York: Springer, 1994.

[17] P. Collings, "Liquid Crystal—Nature's Delicate Phase of Matter (Hilger," England, 1990.

[18] S. Singh and D. A. Dunmur, *Liquid crystals: fundamentals*: World Scientific Publishing Company, 2002.

[19] G. P. Crawford, *Liquid Crystals: Frontiers in Biomedical Applications*: World Scientific, 2007.

[20] J. M. Brake, M. K. Daschner, Y.-Y. Luk, and N. L. Abbott, "Biomolecular interactions at phospholipid-decorated surfaces of liquid crystals," *Science,* vol. 5653, pp. 2094-2097, 2003.

[21] E. Priestley and P. J. Wojtowicz, *Introduction to liquid crystals*: Plenum Publishing Corporation, 1975.

[22] S. V. Shiyanovskii, O. D. Lavrentovich, T. Schneider, T. Ishikawa, Smalyukh, II, C. J. Woolverton, G. D. Niehaus, and K. J. Doane, "Lyotropic chromonic liquid crystals for biological sensing applications," *Molecular Crystals and Liquid Crystals*, vol. 1, pp. 259/[587]-270/[598], 2005.

[23] J. S. Lundgren, A. N. Watkins, D. Racz, and F. S. Ligler, "A liquid crystal pixel array for signal discrimination in array biosensors," *Biosensors and Bioelectronics,* vol. 7, pp. 417-421, 2000.

[24] S. Helfinstine, O. Lavrentovich, and C. Woolverton, "Lyotropic liquid crystal as a real-time detector of microbial immune complexes," *Letters in applied microbiology,* vol. 1, pp. 27-32, 2006.

[25] T. Araki and H. Tanaka, "Surface-sensitive particle selection by driving particles in a nematic solvent," *Journal of Physics: Condensed Matter,* vol. 15, pp. L193, 2006.

[26] L. S. Birchall, R. V. Ulijn, and S. J. Webb, "A combined SPS–LCD sensor for screening protease specificity," *Chemical Communications,* vol. 25, pp. 2861-2863, 2008.

[27] L. A. Tercero Espinoza, K. R. Schumann, Y.-Y. Luk, B. A. Israel, and N. L. Abbott, "Orientational behavior of thermotropic liquid crystals on surfaces presenting electrostatically bound vesicular stomatitis virus," *Langmuir,* vol. 6, pp. 2375-2385, 2004.

[28] G. S. Martin, D. M. Mannino, S. Eaton, and M. Moss, "The epidemiology of sepsis in the United States from 1979 through 2000," *New England Journal of Medicine,* vol. 16, pp. 1546-1554, 2003.

[29] M. Khan, Y. Kim, J. H. Lee, I.-K. Kang, and S.-Y. Park, "Real-time liquid crystal-based biosensor for urea detection," *Analytical Methods,* vol. 15, pp. 5753-5759, 2014.

[30] M. Khan and S.-Y. Park, "Liquid crystal-based proton sensitive glucose biosensor," *Analytical chemistry,* vol. 3, pp. 1493-1501, 2014.

[31] H. Tan, X. Li, S. Liao, R. Yu, and Z. Wu, "Highly-sensitive liquid crystal biosensor based on DNA dendrimers-mediated optical reorientation," *Biosensors and Bioelectronics*, pp. 84-89, 2014.

[32] L.-L. Cheng, Y.-Y. Luk, C. J. Murphy, B. A. Israel, and N. L. Abbott, "Compatibility of lyotropic liquid crystals with viruses and mammalian cells that support the replication of viruses," *Biomaterials,* vol. 34, pp. 7173-7182, 2005.

[33] C. T. Hess, *Skin and Wound Care*: Lippincott Williams & Wilkins, 2008.

[34] M. A. Daeschel and J. McGuire, "Interrelationships between protein surface adsorption and bacterial adhesion," *Biotechnology and Genetic Engineering Reviews,* vol. 1, pp. 413-438, 1998.

[35] D. R. Colman, *Cell Adhesion*: Elsevier Science, 1997.

[36] M. D. Kottke, E. Delva, and A. P. Kowalczyk, "The desmosome: cell science lessons from human diseases," *Journal of cell science,* vol. 5, pp. 797-806, 2006.

[37] M. G. Farquhar and G. E. Palade, "Junctional complexes in various epithelia," *The Journal of cell biology,* vol. 2, pp. 375-412, 1963.

[38] W. A. Wells, "Defining gap junctions," *The Journal of cell biology,* vol. 3, pp. 379-380, 2005.

[39] F. Puoci, *Advanced Polymers in Medicine*, Springer, 2014.

[40] G. Lamour, A. Hamraoui, A. Buvailo, Y. Xing, S. Keuleyan, V. Prakash, A. Eftekhari-Bafrooei, and E. Borguet, "Contact angle measurements using a simplified experimental setup," *Journal of Chemical Education,* vol. 12, pp. 1403-1407, 2010.

[41] M. Żenkiewicz, "Methods for the calculation of surface free energy of solids," *Journal of Achievements in Materials and Manufacturing Engineering,* vol. 1, pp. 137-145, 2007.

[42] K. G. Kabza, J. E. Gestwicki, and J. L. McGrath, "Contact angle goniometry as a tool for surface tension measurements of solids, using zisman plot method. A physical chemistry experiment," *Journal of Chemical Education,* vol. 1, pp. 63, 2000.

[43] H. W. Fox and W. A. Zisman, *Colloid and Science*, pp. 514, 1950.

[44] R. Weast, *Handbook of tables for applied engineering science* vol. The Chemical Rubber Co., Cleveland, Ohio: CRC Press, 1970.

[45] D. Y. Kwok, A. Leung, C. N. C. Lam, A. Li, R. Wu, and A. W. Neumann, "Low-rate dynamic contact angles on poly (methyl methacrylate) and the determination of solid surface tensions," *Journal of colloid and interface science,* vol. 1, pp. 44-51, 1998.

[46] E. G. Shafrin and W. A. Zisman, "Critical surface tension for spreading on a liquid substrate," *The Journal of Physical Chemistry,* vol. 5, pp. 1309-1316, 1967.

[47] D. Y. Kwok and A. W. Neumann, "Contact angle measurement and contact angle interpretation," *Advances in Colloid and Interface Science,* vol. 3, pp. 167-249, 1999.

[48] F. L. Román, J. Faro, and S. Velasco, "A simple experiment for measuring the surface tension of soap solutions," *American Journal of Physics,* vol. 8, pp. 920-921, 2001.

[49] A. W. Adamson and A. P. Gast, "Physical chemistry of surfaces," 1967.

[50] H. Schüring, C. Thieme, and R. Stannarius, "Surface tensions of smectic liquid crystals," *Liquid crystals,* vol. 2, pp. 241-252, 2001.

[51] T. Stoebe, P. Mach, and C. Huang, "Surface tension of free-standing liquid-crystal films," *Physical Review E,* vol. 5, pp. R3587-R3590, 1994.

[52] A. Morita, D. Carastan, and N. Demarquette, "Influence of drop volume on surface tension evaluated using the pendant drop method," *Colloid and Polymer Science,* vol. 9, pp. 857-864, 2002.

[53] T. Beica, R. Moldovan, I. Zgura, and S. Frunza, "Improved Method For Determining The Temperature Dependence Of Interfacial Tension At Liquid Crystal-Isotropic Liquid Interface." Romania: The Publishing House of the Romanian Academy, vol. 7, 2006.

[54] P. R. Griffiths and J. A. De Haseth, *Fourier transform infrared spectrometry*: John Wiley & Sons, 2007.

[55] G. Höhne, W. Hemminger, and H. J. Flammersheim, *Differential scanning calorimetry*: Springer Science & Business Media, 2003.

[56] S. A. Speakman, "Basics of X-Ray Powder Diffraction," *Retrieved June.*

[57] D. Pavia, G. Lampman, G. Kriz, and J. Vyvyan, *Introduction to spectroscopy*: Cengage Learning, 2008.

[58] P. Elmer, "FT-IR Spectroscopy Technical Note," USA.

[59] D. B. Murphy, *Fundamentals of light microscopy and electronic imaging*: John Wiley & Sons, 2002.

[60] C. R. Kokare, "Pharmaceutical Microbiology-Principles and Applications," *Nirali Prakashan. Pune,* 2008.

[61] W. R. Bowen and N. Hilal, *Atomic Force Microscopy in Process Engineering: An Introduction to AFM for Improved Processes and Products* vol.: Butterworth-Heinemann, 2009.

[62] P. C. Braga and D. Ricci, "Atomic force microscopy: application to investigation of Escherichia coli morphology before and after exposure to cefodizime," *Antimicrobial agents and chemotherapy,* vol. 1, pp. 18-22, 1998.

[63] A. Engel and D. J. Müller, "Observing single biomolecules at work with the atomic force microscope," *Nature Structural & Molecular Biology,* vol. 9, pp. 715-718, 2000.

[64] C. K. M. Fung, K. Seiffert-Sinha, K. W. C. Lai, R. Yang, D. Panyard, J. Zhang, N. Xi, and A. A. Sinha, "Investigation of human keratinocyte cell adhesion using atomic force microscopy," *Nanomedicine: Nanotechnology, Biology and Medicine,* vol. 1, pp. 191-200, 2010.

[65] H. X. You, J. M. Lau, S. Zhang, and L. Yu, "Atomic force microscopy imaging of living cells: a preliminary study of the disruptive effect of the cantilever tip on cell morphology," *Ultramicroscopy,* vol. 1, pp. 297-305, 2000.

[66] S. Karrasch, R. Hegerl, J. H. Hoh, W. Baumeister, and A. Engel, "Atomic force microscopy produces faithful high-resolution images of protein surfaces in an aqueous environment," *Proceedings of the National Academy of Sciences,* vol. 3, pp. 836-838, 1994.

[67] C. Müller, M. Allen, V. Elings, A. Engel, and D. J. Müller, "Tapping-mode atomic force microscopy produces faithful high-resolution images of protein surfaces," *Biophysical journal,* vol. 2, pp. 1150-1158, 1999.

[68] P. K. Hansma, J. P. Cleveland, M. Radmacher, D. A. Walters, P. E. Hillner, M. Bezanilla, M. Fritz, D. Vie, H. G. Hansma, C. B. Prater, J. Massie, L. Fukunaga, J. Gurley, and V. Elings, "Tapping mode atomic force microscopy in liquids," *Applied Physics Letters,* vol. 13, pp. 1738-1740, 1994.

[69] S. G. W. Kaminskyj and T. E. S. Dahms, "High spatial resolution surface imaging and analysis of fungal cells using SEM and AFM," *Micron,* vol. 4, pp. 349-361, 2008.

[70] W. Xiao, Q. Wu, X.-t. Yan, and G. Liu, "Application of L293D in main controlling circuit of nurse mobile robot [J]," *International Electronic Elements,* pp. 018, 2007.

[71] T. Thomson, *Design and Applications of Hydrophilic Polyurethanes*: CRC Press, 2000.

[72] A. J. Kinloch, *Adhesion and Adhesives: Science and Technology*: Springer, 1987.

[73] H. Rinck and H. Schneider, "Method of measuring the contact angle of wetting liquid on a solid surface," Google Patents, 1992.

[74] E. G. Shafrin and W. A. Zisman, "Constitutive relations in the wetting of low energy surfaces and the theory of the retraction method of preparing monolayers1," *The Journal of Physical Chemistry*, vol. 5, pp. 519-524, 1960.

[75] C. F. Soon, M. Youseffi, P. Twigg, N. Blagden, and M. C. T. Denyer, "Finite Element Quantification of the Compressive Forces Induced by Keratinocyte on a Liquid Crystal Substrate," in *Analysis and Design of Biological Materials and Structures*: Springer, pp. 79-99, 2012.

[76] J. M. Schakenraad, H. J. Busscher, C. R. H. Wildevuur, and J. Arends, "The influence of substratum surface free energy on growth and spreading of human fibroblasts in the presence and absence of serum proteins," *Journal of biomedical materials research*, vol. 6, pp. 773-784, 1986.

[77] C. F. Soon, M. Youseffi, N. Blagden, R. Berends, S. B. Lobo, F. A. Javid, and M. Denyer, "Characterization and biocompatibility study of nematic and cholesteryl liquid crystals," in *Proceedings of the World Congress on Engineering*, pp. 1872-1875, 2009.

[78] R. N. McElhaney, "The use of differential scanning calorimetry and differential thermal analysis in studies of model and biological membranes," *Chemistry and physics of lipids*, vol. 2, pp. 229-259, 1982.

[79] S. A. Asher and P. S. Pershan, "Alignment and defect structures in oriented phosphatidylcholine multilayers," *Biophysical journal*, vol. 3, pp. 393-421, 1979.

[80] W. Dobbs, L. Douce, and B. Heinrich, "1-(4-Alkyloxybenzyl)-3-methyl-1H-imidazol-3-ium organic backbone: A versatile smectogenic moiety," *Beilstein journal of organic chemistry*, vol. 1, pp. 62, 2009.

[81] C. F. Soon, M. Youseffi, P. Twigg, N. Blagden, and M. C. T. Denyer, "Finite Element Quantification of the Compressive Forces Induced by Keratinocyte on a Liquid Crystal Substrate," in *Analysis and Design of Biological Materials and Structures*., A. Öchsner, L. F. M. Silva, and H. Altenbach, Eds.: Springer Berlin Heidelberg, vol. 14, pp. 79-99, 2012.

[82] I. Kravchenko, Y. Boyko, N. Novikova, A. Egorova, and S. Andronati, "Influence of cholesterol and its esters on skin penetration in vivo and in vitro in rats and mice," *Ukrainica Bioorganica Acta*, vol. 1, pp. 17-21, 2011.

[83] V. A. Ziboh and M. A. Dreize, "Biosynthesis and hydrolysis of cholesteryl esters by rat skin subcellular fractions. Regulation by prostaglandins," *Biochemical Journal*, vol. 2, pp. 281, 1975.

[84] P. G. Unna and L. Goldsdetz, *Biochemical Journal*, vol. 25, pp. 425-431, 1910.

[85] C. F. Soon, S. A. Khagani, M. Youseffi, N. B. Nayan, H. B. Saim, S. Britland, N. Blagden, and M. C. T. Denyer, "Interfacial Study of Cell Adhesion to Liquid Crystals Using Widefield Surface Plasmon Resonance Microscopy," *Colloids and Surfaces B: Biointerfaces*, vol. 110, pp. 2013.

[86] C. F. Soon, W. I. W. Omar, R. F. Berends, N. Nayan, H. Basri, K. S. Tee, M. Youseffi, N. Blagden, and M. C. T. Denyer, "Biophysical characteristics of cells cultured on cholesteryl ester liquid crystals," *Micron*, vol. 56, pp. 73-79, 2014.

[87] J. L. McGrath, "Cell Spreading: The Power to Simplify," *Current Biology*, vol. 10, pp. R357-R358, 2007.

[88] W. I. W. Omar and C. F. Soon, "The Time Based Study of Cell Morphology Using Atomic Force Microscopy," in *5th International Conference on Biomedical Engineering in Vietnam*, pp. 227-230, 2015.

[89] D. E. Discher, P. Janmey, and Y.-l. Wang, "Tissue cells feel and respond to the stiffness of their substrate," *Science*, vol. 5751, pp. 1139-1143, 2005.

[90] B. S. Mitchell, *An Introduction to Materials Engineering and Science for Chemical and Materials Engineers*: John Wiley & Sons, 2004.

[91] C. F. Soon, M. Youseffi, N. Blagden, and M. C. T. Denyer, "Characterizing the interfacial topology of cells attached to liquid crystals," in *Biomedical Engineering and Sciences (IECBES), 2012 IEEE EMBS*, pp. 252-255, 2012.

[92] R. Jahn, T. Lang, and T. C. Südhof, "Membrane Fusion," *Cell*, vol. 4, pp. 519-533, 2003.

[93] A. Engler, L. Bacakova, C. Newman, A. Hategan, M. Griffin, and D. Discher, "Substrate Compliance versus Ligand Density in Cell on Gel Responses," *Biophysical journal*, vol. 1, pp. 617-628, 2004.

[94] D. S. Katti, R. Vasita, and K. Shanmugam, "Improved biomaterials for tissue engineering applications: surface modification of polymers," *Current topics in medicinal chemistry*, vol. 4, pp. 341-353, 2008.

[95] E. A. Cavalcanti-Adam, T. Volberg, A. Micoulet, H. Kessler, B. Geiger, and J. P. Spatz, "Cell Spreading and Focal Adhesion Dynamics Are Regulated by Spacing of Integrin Ligands," *Biophysical journal,* vol. 8, pp. 2964-2974, 2007.

[96] V. D. C. Shields, "High resolution ultrastructural investigation of insect sensory organs using field emission scanning electron microscopy," *Microscopy: Science*, *Technology*, *Applications and Education*, pp. 321-328, 2010.